23

STREPTOMYCIN RESISTANCE: A GENETICALLY RECESSIVE MUTATION

JOSHUA LEDERBERG

Department of Genetics, College of Agriculture, University of Wisconsin, Madison, Wisconsin

Reprinted from JOURNAL OF BACTERIOLOGY
Vol. 61, No. 5, May, 1951

Made in United States of America

Reprinted from JOURNAL OF BACTERIOLOGY
Vol. 61, No. 5, May, 1951

STREPTOMYCIN RESISTANCE: A GENETICALLY RECESSIVE MUTATION

JOSHUA LEDERBERG

Department of Genetics,[1] *College of Agriculture, University of Wisconsin, Madison, Wisconsin*

Received for publication February 2, 1951

The spontaneous mutation of bacteria from their normal status of streptomycin sensitivity (S^s) to resistance (S^r) is of considerable importance in the clinical application of this chemotherapeutic agent (Miller and Bohnhoff, 1950) as well as in experimental studies of bacterial variation. An important datum hitherto lacking is the dominance relationship of S^s and S^r; that is, whether the presence of S^s and S^r together in the same cell results in sensitivity or in resistance.

This question has been studied with the help of unique heterozygous diploid cultures of *Escherichia coli*. These cultures have been isolated from crosses of polyauxotrophic mutants of strain K-12. A detailed account of the isolation techniques and of the genetic behavior that led to the characterization of these cultures as heterozygous diploids has been published (Lederberg, 1949; Zelle and Lederberg, 1951). The same considerations identify the present material as diploids carrying an S^s and S^r factor from each parent.

The parent stocks used to obtain diploid cultures heterozygous for S^s/S^r were W-67 and W-1177. W-67 is auxotrophic for biotin and methionine (double mutant stock 58-161; Tatum, 1945) and also carries a lactose-negative mutation, Lac_4-, but is the wild type or is sensitive to streptomycin. W-1177 (677 sr; Lederberg, 1950) is auxotrophic for threonine, leucine, and thiamine; lactose- and maltose-negative; and S^r. The two parents can be symbolized as $B-\ M-\ T+\ L+\ B_1+\ Lac_1+\ Lac_4-\ S^s$ and $B+\ M+\ T-\ L-\ B_1-\ Lac_1-\ Lac_4+\ S^r$, respectively. They were crossed on a synthetic EMB lactose agar medium, and the rare lactose-positive prototrophs were isolated to verify whether they were diploid heterozygotes ($Lac_1+\ Lac_4-/Lac_1-\ Lac_4+$) or recombinant prototrophs ($Lac_1+\ Lac_4+$). (See Lederberg, 1949, p. 183.) From such crosses, two diploid cultures were isolated that were heterozygous for S^r/S^s. These were purified by repeated single colony isolations on synthetic EMB lactose agar plates.

Suspensions of the diploid cultures were grown in aerated synthetic liquid medium containing lactose. From 90 to 95 per cent of the cells from such cultures were still diploid. The remainder consists of haploid segregants which are continually thrown off by the diploid cells but which are incapable of sustained growth in the synthetic medium owing to the auxotrophic and lactose-negative mutations that they have inherited from the original parents. The following report of the platings of the diploid suspensions is corrected for the segregants initially present.

[1] Paper No. 450. Supported in part by a grant from the Research Committee of the University of Wisconsin, with funds from the Wisconsin Alumni Research Foundation.

The suspensions were diluted 10^{-6}, and 0.1-ml samples that contained about 100 viable cells were spread on the surface of complete EMB lactose agar plates (made up with peptone and yeast extract), to which varying quantities of streptomycin had been added. The plates were then incubated at 37 C for 24 to 48 hours. The two S^r/S^s diploids behaved in the same way. The diploid cells failed to form colonies on agar containing 2 or more micrograms of streptomycin per ml. This was likewise the threshold concentration for complete inhibition of K-12, W-67, and S^s diploids from other crosses. At lower levels, from 0.5 to 2 micrograms, a variable proportion of the diploid cells formed colonies. The appearance of these colonies on EMB lactose agar clearly showed that haploid segregants had largely outstripped the growth of the initial diploid cell, and this was verified by tests of the bacteria making up the colonies. This concentration range is likewise that over which the growth of control S^s colonies is partially inhibited. On the other hand, S^r cultures, whether haploid or diploid, were unaffected at the highest level tested, 500 micrograms per ml.

It is clear that S^s is fully dominant to S^r.

It must be noted that only a few of the diploids isolated from the cross of W-67 × W-1177 proved to be heterozygous for the S factors. This and related anomalies will be documented more thoroughly elsewhere, but there is no reason to suspect that they would have any influence on the dominance tests related here.

Because of the perceptible occurrence of many presumably recessive mutations, most bacteria are generally regarded as haploid. To the extent that this supposition is correct, dominance relationships will not be grossly important in the development of bacterial resistance to antibiotics. However, there are compelling suggestions that many bacteria are multinucleate, although the nuclei probably sort out in a few fissions. The dominance of S^s would then have the effect of delaying the initiation of the expression of S^r mutations for these few fissions (compare Newcombe and Hawirko, 1949).

REFERENCES

LEDERBERG, J. 1949 Aberrant heterozygotes in *Escherichia coli*. Proc. Natl. Acad. Sci. U. S., **35**, 178–184.

LEDERBERG, J. 1950 The selection of genetic recombinations with bacterial growth inhibitors. J. Bact., **59**, 211–215.

MILLER, C. P., AND BOHNHOFF, MARJORIE 1950 The development of bacterial resistance to chemotherapeutic agents. Ann. Rev. Microbiol., **4**, 201–222.

NEWCOMBE, H. B., AND HAWIRKO, ROMA 1949 Spontaneous mutation to streptomycin resistance and dependence in *Escherichia coli*. J. Bact., **57**, 505–572.

TATUM, E. L. 1945 X-ray induced mutant strains of *Escherichia coli*. Proc. Natl. Acad. Sci. U. S., **31**, 215–219.

ZELLE, M. R., AND LEDERBERG, J. 1951 Single-cell isolations of diploid heterozygous *Escherichia coli*. J. Bact., **61**, 351–355.